THE MEASUREMENT OF BACTERIA.

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Bacteria are generally measured by means of the micrometer eyepiece, which contains a scale graduated into divisions. The values of these divisions are actually determined for the various objectives used by the microscopist by focussing the scale of a stage-micrometer and noting the number of ocular divisions included in a certain number of $\frac{1}{100}$ millimetre divisions of the stage-micrometer scale. The value of a single division is then calculated, and is thus a known constant for the objective with a certain tube length.

In measuring bacteria it is usual to employ the micrometer eyepiece and the $\frac{1}{12}$ oil-immersion objective, with a tube length advised by the maker of the objective. On no account should the value of a micrometer division be assumed or accepted without personal confirmation. For instance, the values of the divisions with a Leitz $\frac{1}{12}$ oil-immersion, micrometer eyepiece ii. and tube length 170 mm., by actual determination was found to be equal to $1{\cdot}5\,\mu$; according to Leitz's price list, it is $1{\cdot}8\,\mu$.

With a micrometer eyepiece the unit of measurement of which equals say $1{\cdot}5\,\mu$, the measurement of bacteria is uncertain unless the boundaries of the organisms coincide with the divisional lines. Fractions of the unit ($1{\cdot}5\,\mu$) necessitate an estimation, and it is here that the uncertainty occurs, for the eye cannot divide a small space into 10 or 15 equal parts. Errors of measurement frequently happen. As far as the length is concerned, this is of little consequence, because on a film the bacteria are found in

lengths varying between the normal length of a mature cell and twice that length when the cell is about to divide into two individuals. Small bacteria may even appear less than the normal when they are lying in the film at an angle with the coverglass. It is of greater consequence to have a correct estimation of the breadth of bacteria, and especially the breadth in relation to the normal length, for then we can have a true picture of the organism. Small differences in breadth influence the general appearance of the cells to a greater degree than small differences in length. This can be clearly seen by comparing the two figures, in one of which (fig. 1) the diagrammatic bacteria have a constant breadth and varying length, and in the other (fig. 2) they have a constant length and varying breadth.

Fig. 1.

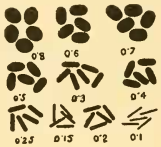

Fig. 2.

Since, owing to the method of reproduction of the fission fungi, the breadth is more constant than the length, all suggestions for arriving at a truer approximation of the breadth than can be made by estimating it directly with the micrometer eyepiece, should be welcomed. Means other than the convenient micrometer eyepiece have been advised. Wright* proposed to project the image of a scale or of a system of squares upon the plane upon which the microscopic objects are disposed. Wilson and Randolph† photograph the bacteria at a magnification of 1000 diameters. They also photograph a ruled system of squares so that the rulings are exactly one millimetre apart. Then by

* Journ. Roy. Microscop. Soc., 1897, 182.

† Journ. Applied Microscopy, 1899, 598.

double printing they obtain an image of the bacteria upon a network of squares. This method is good for recording the measurements in a pictorial manner, and for actually measuring the breadth it is better than the micrometer eyepiece, inasmuch as the unit of size becomes 1 μ instead of 1·5 or 1·8 μ. For exact measurement, it would be easy, once the bacteria were photographed, to place the negative under a low power objective and measure the breadth with the micrometer eyepiece, or to project the image upon a screen by means of the projection lantern and measure with a centimetre or millimetre rule. This of necessity involves photographing the organism, a process which is not always desired.

The method I employ in determining the breadth of an organism is to fix upon a bacterium in the microscopic field and measure its length. Then I compare the organism with a series of diagrams representing bacteria, the breadths of which have been accurately measured in terms of the length. From this series one group that appears identical with the organism fixed upon is noted, and the number of this group is multiplied by the length of the organism. The result is the breadth. The breadth of another organism in the same film may be calculated in a similar manner, and the second result will generally be identical with the first. For example, the organism is a short rod measuring 1·5 μ, and on comparison with the diagrammatic table it appears identical with the group whose type number $(\frac{\text{breadth}}{\text{length}})$ is 0·4. On multiplying 1·5 μ by 0·4, the breadth 0·6 μ is obtained. This result will be more exact than that obtained by estimating the breadth with the micrometer eyepiece. Since this estimation of the breadth when done from a longer and a shorter form in the same film necessitates two different calculations, these, when they agree, are more likely to be correct than when several are estimated by a similar mental estimation as obtains when the eyepiece micrometer alone is used. It, however, goes without saying that it is advisable to check the one method against the other.

A large diagram of types may be employed, but perhaps a better idea is obtained when the types are reduced to sizes approximating those observed with the oil-immersion. These are given in the accompanying figure (fig. 3), where the long rods measure three millimetres and the shorter rods 1·2 mm.

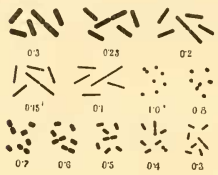

Fig. 3.

The diameters of micrococci, streptothrix and other forms might be confirmed after micrometer measurement by comparing the coccus, etc., relative to the micrometric scale division lines, with lines ruled at intervals of 1·2 mm. (the length of the smaller diagrammatic organisms) upon a coverglass which is superposed over the shorter diameter of the diagrammatic types. If the coccus or streptothrix occupies a space in the divisions of the eye-piece similar to that occupied by one of the types when viewed under such a ruled coverglass, it is obvious that the diameter of the coccus, etc., will be the type number multiplied by the value of the micrometer divisions. Such rulings can be made upon a coverglass by dipping the latter into a dilute solution of gelatine (0·5 %) and ruling the lines with Indian ink upon the thin dry gelatine film.

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